Vol. 115, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 15, 1983 Pages 598-605

COMPETENCE AND PROGRESSION GROWTH FACTORS STIMULATE DIFFERENT tRNA^{lys} MODIFICATION REACTIONS IN BALB/C 3T3 CELLS

Victor K. Lin and B.J. Ortwerth

Departments of Biochemistry and Ophthalmology
University of Missouri Health Sciences Center
Columbia, MO 65211

Received August 1, 1983

SUMMARY. Sparse cultures of Balb/C 3T3 cells were growth arrested in a medium containing 1% calf plasma. This treatment caused a decrease in tRNA$_4^{lys}$, a tRNA species related to cell division, and a corresponding increase in tRNA$_5^{lys}$. A restoration of tRNA$_4^{lys}$ levels and an increase in cell number was obtained by the addition of either 10% calf serum or a combination of fibroblast growth factor and melanocyte stimulating activity. Neither growth factor alone evoked a proliferative response. The progression factors, insulin and multiplication stimulating activity stimulated the rapid conversion of tRNA$_5^{lys}$ to tRNA$_2^{lys}$, whereas the competence factors, fibroblast growth factor and platelet-derived growth factor stimulated a slight conversion of tRNA$_2^{lys}$ to tRNA$_4^{lys}$.

The culturing of most mammalian cells in vitro requires the presence of serum or growth factors in the culture medium to support cell viability and stimulate cell proliferation (1,2). Pledger et al (3) have shown that the mitogenic response to serum can be duplicated by the simultaneous addition of platelet derived growth factor (PDGF) and plasma. An ordered sequence of events during this mitogenic response has been demonstrated by Stiles et al. (4) and they postulated that there are two sets of growth factors controlling different phases of the cell cycle. Competence factors, including PDGF and FGF, induce Balb/C 3T3 cells to become competent to synthesize DNA, whereas progression factors, including somatomedins, MSA and insulin, allow competent cells to progress through G_0/G_1 and enter S phase.

Our laboratory has studied lysine tRNA changes in mammalian cells under different growth conditions. Lysine tRNA from mammalian cells contains from 3

ABBREVIATIONS

MSA, multiplication stimulating activity; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; DME, Dulbecco's modified Eagle's medium; t^6A, threonyl carbamoyl adenosine.

0006-291X/83 $1.50

to 9 isoaccepting species all of which respond to either AAA or AAG condons (5-7). One of these isoaccepting species, $tRNA_4^{lys}$, has been suggested to have a role in mammalian cell proliferation as the level of this tRNA correlates exactly with the growth rate of cells in culture (8,9). Furthermore, the level of this tRNA remains high when cells are rapidly dividing, but, when cell division is inhibited, there is a decrease in $tRNA4^{lys}$ prior to the inhibition of proliferation. We have suggested that these alterations in $tRNA^{lys}$ isoaccepting species may result from a growth factor mediated control of different $tRNA^{lys}$ modification enzymes.

MATERIALS AND METHODS. Balb/C3T3 cells, clone A31 were obtained from the American Type Culture Collection and propagated in DME with 10% calf serum. Sterile bovine plasma was obtained from Pel-Freeze Biologicals. FGF, PDGF and MSA were purchased from Collaborative Research Inc. and insulin from Sigma Chemical Co.

Cell Growth. Cells were grown for several days in 35mm dishes from an innoculum of $2x10^4$ cells per dish. The medium was changed to DME with 1% calf plasma, after 48 hours various growth factors or 10% serum were added directly to the medium and cell number was determined after an additional 48 hours. Each point was determined by trypsinization of triplicate dishes and counting in a Coulter cell counter.

Lys-tRNA Profiles. Cells were grown in 150mm dishes from an innoculum of $3.6x10^5$ cells per dish and continued in a manner identical to the cell growth experiment. RNA was isolated from the cells by direct phenol-buffer extraction of the plates and tRNA was isolated by DEAE cellulose chromatography. Each sample of tRNA was aminoacylated with [^{3}H]lysine, isolated over a DEAE cellulose column and fractionated by RPC-5 chromatography. These procedures have been described in detail previously (9).

RESULTS

Subconfluent cultures of Balb/C 3T3 cells were transferred from DME medium containing 10% calf serum to DME medium containing 1% calf plasma and allowed to remain in this serum-deficient medium for 48 hours to exhaust the endogenous growth factors. As predicted, the growth of cells was inhibited under these conditions as shown in Figure 1. The cell number remained constant for the first day then decreased continuously over the next 3 days in the 1% plasma medium while the cells in the 10% serum medium continued to grow until they reached confluency. The addition of serum restored cell proliferation as the cells doubled over the next 48 hours. Purified FGF, a competence factor, and MSA, a progression factor, when added together restored the proliferative ability of the cells almost as well as that of 10% calf serum. Purified FGF or PDGF alone may have had a moderate effect on cell proliferation as a slight

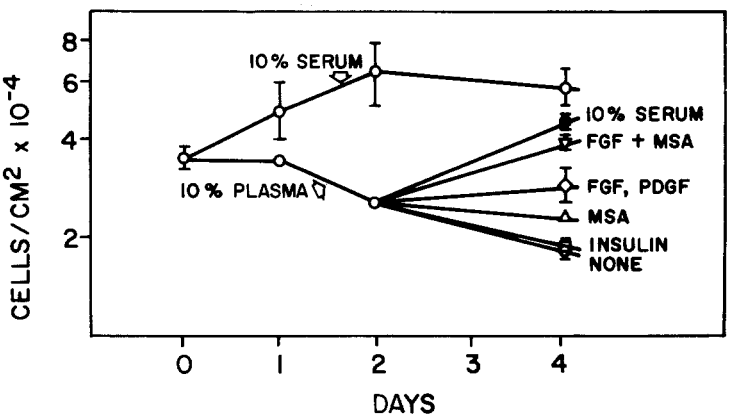

Figure 1 - The growth of Balb/C 3T3 cells in response to bovine plasma, calf serum and purified growth factors.

Cells were placed in medium containing 1% calf plasma (open circles) for 48 hours at which time either 10% calf serum (closed circles), 20ng/ml FGF (diamond), 0.5 unit/ml PDGF (diamond), 200mg/ml insulin (squares), 67ng/ml MSA (triangles) or 20ng/ml FGF and 67ng/ml MSA (inverted triangles) was added to the cultures without a medium change. Cell numbers were determined at the times shown.

increase in cell number was seen. The addition of either insulin or MSA alone did not result in a net increase in cell number over that seen at day 2. Clearly both growth factors were required to restore cell division in this system.

Figure 2 shows a typical RPC-5 profile of [^{3}H]lys-tRNA from Balb/C 3T3 cells in log growth. The numbering system employed here and in previous work (5,8) is also shown. Companion cultures to the ones used to determine cell number in Figure 1 were also extracted and the lysyl-tRNA profiles were determined. The radioactivity in each peak was determined and calculated as the percent of the total lysyl-tRNA recovered from the column. The recovery in each case was 90-100% of the applied sample (1-5x10^4 cpm). The changes in $tRNA^{lys}$ profiles during growth arrest in 1% calf plasma are shown in Table I. A significant increase in the relative amount of $tRNA_5^{lys}$ combined with decreases in $tRNA_2^{lys}$ and $tRNA_3^{lys}$ and $tRNA_4^{lys}$ was observed within 24 hours. The $tRNA^{lys}$ profiles in 1% calf plasma were identical to those we had observed in confluent Balb/C 3T3 cells (7).

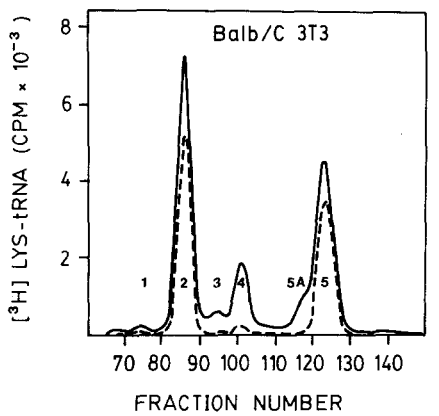

Figure 2 - RPC-5 chromatographic profile of sparse Balb/C 3T3 cell [^{3}H]lys-tRNA (———) and rat liver [^{14}C]lys-tRNA (---).

The effects of purified growth factors on $tRNA^{lys}$ profiles are shown in Figure 3. 10% serum and the combination of FGF plus MSA caused a decrease in $tRNA_5^{lys}$ within one hour along with increases in $tRNA_2^{lys}$, $tRNA_3^{lys}$ and $tRNA_4^{lys}$. These additions, therefore, caused a complete and rapid reversal of the effects of 1% plasma, suggesting an alteration in tRNA modification. With time there was a subsequent decrease in $tRNA_2$ and an increase in $tRNA_4^{lys}$ to 10% of the total, which was higher than at the start of the experiment. Significantly it was these conditions which caused a cell doubling after48 hours later.

The addition of either FGF or PDGF alone did not cause any immediate decrease in $tRNA_5^{lys}$. At 24 hours, however, $tRNA_5^{lys}$ did decrease, possibly due to small amounts of residual progression factors in the 1% plasma medium. At 1 and 6 hours FGF and PDGF did cause a decrease in $tRNA_2^{lys}$ and a small increase in $tRNA_4^{lys}$ and a slight increase in cell number was seen 48 hours

TABLE I

Changes in $tRNA^{lys}$ profiles of Balb/C 3T3 cells in medium with 1% plasma

DAYS IN 1% PLASMA	$tRNA_2^{lys}$	$tRNA_3^{lys}$	$tRNA_4^{lys}$	$tRNA_{5+5A}^{lys}$
	(Percent of Total)			
0	51	6.6	7.7	34
1	48	2.5	5.7	43
2	49	1.9	3.6	44
3	46	1.0	4.0	49

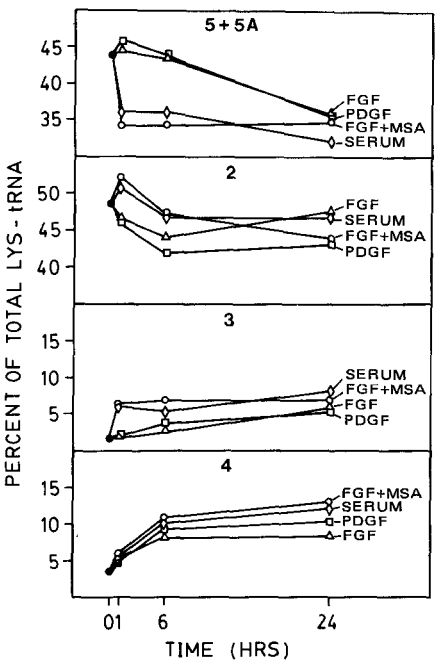

Figure 3 - Changes in the distribution of Balb/C 3T3 cell [^{3}H]lys-tRNA in response to calf serum and purified competence factors.

Cells were treated as described in Fig. 1 and the [^{3}H]lys-tRNA profiles were determined at 1, 6 and 24 hours after the addition of 10% calf serum (diamonds), 20ng/ml, FGF (triangles), 0.5unit/ml PDGF (squares), or 20ng/ml FGF and 67ng/ml MSA (circles). The numbers above each graph represents the number of the $tRNA^{lys}$ species measured.

later. Therefore the enzyme which converts $tRNA_2^{lys}$ to $tRNA_4^{lys}$ appears to be activated by purified competence factors, but both progression and competence factors were required for the complete conversion of $tRNA_5^{lys}$ to $tRNA_4^{lys}$.

Figure 4 shows the effect of MSA and insulin on $tRNA_5^{lys}$ profiles. Both progression factors alone caused a rapid decrease in $tRNA_5^{lys}$, but a greater effect was seen with both MSA and FGF. $tRNA_2^{lys}$ increased slightly with each treatment. The subsequent decrease in $tRNA_2^{lys}$ and increase in $tRNA_3^{lys}$ and $tRNA_4^{lys}$, however, was diminished in the absence of FGF. Therefore progression factors appear to stimulate the modification of $tRNA_5^{lys}$ to $tRNA_2^{lys}$, but the presence of competence factors are required for the rapid conversion of $tRNA_2^{lys}$ to $tRNA_3^{lys}$ and $tRNA_4^{lys}$.

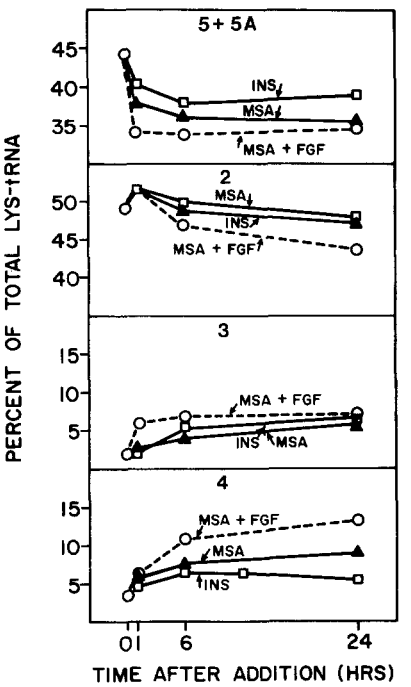

Figure 4. Changes in the distribution of Balb/C 3T3 cell [^{3}H]lys-tRNA profiles in response to the addition of calf serum or purified progression factors.

Cells were treated as described in Fig. 1 and the [^{3}H]lys-tRNA profiles were determined at 1,6 and 24 hours after the addition of 200ng/ml insulin (squares), 67nm/ml MSA (closed triangles) or 20ng/ml FGF and 67ng/ml MSA (circles).

After several months in continuous culture, these cells failed to show the increase in $tRNA_5^{lys}$ due to growth in 1% plasma. These cells were subsequently found to grow to a confluent density of $5x10^5/cm^2$ and therefore had lost much of their contact inhibited character. Cells taken from an earlier freeze responded as the original cells and $tRNA^{lys}$ responses to growth factors were similar at 1 and 6 hours which were the only times studied.

DISCUSSION. The results presented here confirm the need for at least two growth factors to stimulate proliferation in Balb/C 3T3 cells (4). Two examples from each growth factor class were used and similar responses in $tRNA^{lys}$ modification were seen in both cases. The $tRNA^{lys}$ response occurs rapidly, suggesting changes in tRNA modification enzymes as opposed to new tRNA synthe-

sis. The time frame is also consistent with the idea that $tRNA_4^{lys}$ synthesis is an early G1 event.

The $tRNA^{lys}$ changes reported here are identical to those obtained from trypsinized, confluent cells (8). The data suggest a $tRNA^{lys}$ modification pathway from $tRNA_5^{lys}$ to $tRNA_2^{lys}$ to $tRNA_4^{lys}$, with $tRNA_3^{lys}$ as a possible intermediate. Cells which did not show an increase in $tRNA_5^{lys}$ did not respond to growth factors. Therefore the normal $tRNA_5^{lys}$ species may not be a substrate for the growth factor-stimulated, modification enzymes. Rather a $tRNA_4^{lys}$-related tRNA species, if not fully modified, may chromatograph with $tRNA_5^{lys}$ and serve as the substrate for these enzymes.

These data are of interest because two different classes of growth factors mediate two different enzymes in an apparent pathway leading to the synthesis of $tRNA_4^{lys}$, a species which has been correlated directly with the rate of cell division in untransformed cell lines. Continued studies on the tRNA modification enzymes are warranted as these enzymes 1) appear to act in a specified modification sequence 2) act on mature tRNAs which can be aminoacylated in vitro 3) recognize homologous tRNAs as substrates and 4) appear to be controllable enzymes.

It is now clear that one of the major responses of a cell to growth factors is the activation of specific protein kinases (10,11). It is interesting that in the $tRNA^{lys}$ system there is potential for control by phosphorylation of both enzymes and tRNA. The hypermodified nucleotide in this tRNA family is t^6A. Sequencing studies by Raba et al. (12) on $tRNA_4^{lys}$ from SV-40 transformed 3T3 cells and by Hedgcoth and Ortwerth (unpublished results) on $tRNA_4^{lys}$ from mouse leukemic cells show the presence of an altered t^6A residue in both tRNAs located next to the anticodon sequence.

ACKNOWLEDGEMENT

This work was supported by grant CA 26423 from the National Cancer Institute and by the Eye Research Foundation of Missouri Inc.

REFERENCES

1. Holley, R.W. and Kiernan, J.A. (1974) Proc. Natl. Acad. Sci. USA 71:2908-2911.
2. Barnes, D. and Sato, G. (1980) Anal. Biochem. 102:255-270.

3. Pledger, W.J., Stiles, C.D., Antonaides, H.N. and Scher, C.D. (1977) Proc. Natl. Acad. Sci. USA 74:4481-4485.
4. Stiles, C.D., Capone, G.T., Scher, C.D., Antonaides, H.N., Van Wyk, J.J. and Pledger, W.J. (1979) Proc. Natl. Acad. Sci. USA 76:1279-1283.
5. Juarez, H., Juarez, D. and Hedgcoth, C. (1974) Biochem. Biophys. Res. Commun. 61:171-178.
6. Bjercke, R.J. and Hedgcoth, C. (1981) Mol. Gen. Genet. 183:528-531.
7. Ortwerth, B.J., Yonuschot, G.R. and Carlson, J.V. (1973) Biochemistry 12:39853991.
8. Conlon-Hollingshead, C. and Ortwerth, B.J. (1980) Exp. Cell Res. 128:171-180.
9. Ortwerth, B.J., Wolters, J., Nahlik, J. and Conlon-Hollingshead, C. (1982) Exp. Cell Res. 138:241-250.
10. Nishimura, J., Huang, J.S. and Deuel, T.F. (1982) Proc. Natl. Acad. Sci. USA 79:4303-4307.
11. Kasuga, M., Fujita-Yamaguchi, Y., Blithe, D.L. and Kahn, R. (1983) Proc. Natl. Acad. Sci. USA 80:2137-2141.
12. Raba, M., Limburg, K., Burghagen, M., Katze, J.R., Simsek, M., Heckman, J.E., Rajbhandary, U.L. and Gross, J.H. (1979) Eur. J. Biochem. 97:305-318.